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Second-Order Model to Predict Microbial Degradation of Organic Compounds in Natural Waters

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16. ABSTRACT

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Second-Order Model to Predict Microbial Degradation of Organic Compounds in Natural Waters

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The reliability of second-order rate constants for assessing microbial degradation kinetics in natural waters was examined by using three compounds that undergo hydrolytic degradation. The butoxyethyl ester of 2,4-dichlorophenoxyacetic acid was studied in water samples from 31 sites, malathion was examined in water from 14 sites, and chlorpropham was studied in samples from 11 sites. The coefficient of variation for rate constants for each compound was less than 65% over all sites. Additional studies indicated that the rate conformed to second-order kinetics; that is, the rate was proportional to both bacterial and xenobiotic concentrations.

Predicting the role of microorganisms in controlling the fate and concentration of xenobiotics in natural waters requires a reliable mathematical description of process kinetics. For maximal use, this kinetics description should incorporate parameters that allow prediction of rates in a wide variety of environments. Even if they were not highly accurate, relationships of this type would be of great utility in predicting the expected environmental behavior of a pollutant.

It is well known that environmental properties such as pH, microbial population, water temperature, and the natural characteristics of the xenobiotic will influence the fate of a chemical in the aquatic environment. Unfortunately, the fact that such properties can cause variable behavior has usually been invoked to demonstrate the futility of the kinetics approach to pollutant transport and fate. The result is that few rate constants have ever been measured in natural waters or with indigenous populations.

In earlier laboratory studies (7) in which bacteria were cultured in a defined medium with the xenobiotic as the sole carbon source, the rate of removal of xenobiotics from the medium was described by the modified Monod expression (11)

$$-\frac{d[S]}{dt} = \frac{\mu_m[S][B]}{Y(K_s + [S])} \quad (1)$$

where $[S]$ is the concentration of xenobiotic (milligrams/liter), $[B]$ is the concentration of bacteria (organisms/liter), μ_m is the maximal growth rate (per hour), Y is the yield factor or number of bacteria produced per milligram of xenobiotic, and K_s is the constant numerically equal to the xenobiotic concentration at which $\mu = \frac{1}{2}\mu_m$.

When the substrate concentration is much higher than K_s, then equation 1 is reduced to

$$-\frac{d[S]}{dt} = \frac{\mu_m}{Y}[B] \quad (2)$$

The rate of degradation is zero order with substrate concentration and first order with cell concentration.

When the substrate concentration is much lower than K_s, then equation 1 is reduced to

$$-\frac{d[S]}{dt} \simeq \frac{\mu_{max}}{YK_s}[B][S] \simeq k[B][S] \quad (3)$$

where k is a second-order rate constant (liters per organism per hour) for removal of substrate by bacteria. Although in natural waters the xenobiotics would not be the sole carbon source, our hypothesis is that a second-order approach can be used to describe microbial transformation of the xenobiotics.

In this study, we report on the application of a second-order kinetics approach to describe the transformation of xenobiotics by microorganisms and on the site-to-site reproducibility of the second-order rate constants.

MATERIALS AND METHODS

Water collection. Four to eight liters of water was collected from 40 sites in 18 states. Water temperatures were recorded at the time of collection. The samples were transported in sterile glass jugs and stored in refrigerators (7°C) at the laboratory. Studies of process kinetics were made within 48 h of receipt at the laboratory; sequential studies were made within 30 days.

At the laboratory, hydrogen ion activity was determined with a Beckman Zeromatic pH meter; total organic carbon was quantified with the Beckman total carbon analyzer model 915; nitrogen, phosphorus, and hardness were determined by using the Limnology Kit of Ecologic Instrument Corp.; salinity was determined

with a temperature-compensated American Optical refractometer.

Bacterial concentrations were determined by plate counts, using tryptone glucose extract agar and marine agar (Difco Laboratories). Plates were incubated for 48 h at 22°C and counted on a Quebec colony counter. Major genera of bacteria present in the fresh waters were identified according to the scheme of Skerman (9). Commercially prepared tests (Oxi/Ferm tubes, Enterotubes, and Micro I.D.) were used to aid in some of the bacterial identifications.

Chemicals. Malathion (99% purity) was purchased from Teklab, Inc., and used as received. The butoxyethyl ester of 2,4-dichlorophenoxyacetic acid (2,4-DBE) was purchased from Amchem Co. and used as received. Chlorpropham (CIPC; Pittsburgh Plate Glass Co.) was recrystallized three times from absolute alcohol and diethyl ether (mp 38.0 to 38.5°C). Organic solvents used were pesticide grade, distilled in glass (Burdick and Jackson, Inc.).

A saturated solution of each compound was prepared by stirring the compound into distilled water without carrier and sterilized by passing the solution through a sterile 0.22-μm membrane filter (Millipore Corp.). The filtrate was used as the stock solution for spiking the natural waters. To avoid a 10% dilution of nutrients in Overlook Lake samples, sterile water from the lake was used for the stock solution to provide the required concentration of 1.0 mg of 2,4-DBE per liter.

Design of kinetic degradation experiments. Approximately 200 ml of each sample was transferred to five 500-ml flasks. To four of these, a filter-sterilized aqueous solution of the test compound was added to give a final concentration of 0.1 mg of compound per liter in two flasks and 0.01 mg per liter in another two flasks for malathion and 2,4-DBE. For ease of analysis, concentrations of 1.0 and 0.1 mg/liter were used for CIPC. The remaining flask without pesticide served as a control to determine any changes in bacterial populations not due to presence of the xenobiotics. Another flask containing 200 ml of filter-sterilized natural water containing the compound served as a control for abiotic processes. Flasks were incubated in a temperature-controlled shaker; the temperature was adjusted to coincide with the temperature of the water when collected. The sampling schedule was arranged so that at least 10 to 12 samples were collected through at least 2 half-lives of the parent compound. Each time a sample was taken for compound analysis, a sample was taken for bacterial count. Bacteria levels and compound concentrations were monitored with time by plate counts and gas-liquid chromatography, respectively. At the end of the experiments, organic extracts of the flask contents were analyzed by thin-layer chromatography to determine the predominant products.

Overlook Lake samples used for studies to determine the kinetic order of degradation had bacterial concentrations of 10^9 organisms/liter. Sufficient quantities of water were collected to concentrate the bacteria 10- and 100-fold by centrifugation. Approximately 100 ml of sample water per 250-ml flask was used instead of 200 ml as for the other experiments.

Extraction and chromatography. All analyses were performed with a Tracor MT-220 gas-liquid chromatograph equipped with a ^{63}Ni high-temperature electron capture detector. Nitrogen was the carrier gas at a flow of 80 ml/min; operating temperatures for the inlet port and detector were 225 and 300°C, respectively. The column was 1.2 m by 4 mm (inside diameter) and packed with 3% SE-30 on 80- to 100-mesh Gas Chrom Q (Supelco Co.). Compounds were extracted from water samples with 2,2,4-trimethylpentane (isooctane), and no sample clean-up was needed.

Water samples containing malathion were adjusted to pH 2 with 1.0 N hydrochloric acid, extracted with methylene chloride, and analyzed by thin-layer chromatography (silica gel) with hexane-acetic acid-ethyl ether (75:15:10) (3) as the developing solvent. Compounds were revealed by spraying the plate with the reagent of Menn et al. (6), 0.5% 2,6-dibromo-*N*-chloro-*p*-quinoneimine in acetone. R_f values of the product were compared with R_f values of the standards.

For 2,4-DBE studies, the water samples were acidified with 6 N hydrochloric acid, extracted with diethyl ether, and evaporated. Thin-layer chromatography was done by using alumina with carbon tetrachloride-hexane-acetic acid (75:10:15) as the developing solvent and Kovacs reagent (5) for visualization.

CIPC water samples were extracted with methylene chloride; the solvent system was benzene-acetic acid (4:1). Glass plates coated with silica gel containing a fluorescent reagent were used, and detection of components was done with ultraviolet light.

Degradation kinetics. Pseudo-first-order rate constants ($k[B]$) were obtained from a least-squares analysis. Second-order rate constants (k) were obtained by dividing $k[B]$ by the bacterial concentration ($[B]$). Bacterial concentrations did not change significantly during the experiments; therefore, the values obtained for a bacterial population during an experiment were averaged, and this value was used in the model for $[B]$.

RESULTS

Determination of kinetic order for compound degradation in natural waters. Samples for these studies were taken during the summer from Overlook Lake in north Georgia. The time that this collection was made was different from that for the sample used for site-to-site studies reported in Table 3. The bacterial concentration for Overlook Lake in the site-to-site study was 9.8×10^8 organisms/liter, whereas for this study the bacterial concentration was 2.5×10^9 organisms/liter in the water collected. 2,4-DBE and bacterial concentrations were varied over three orders of magnitude. Compound concentrations were 0.01, 0.1, and 1.0 mg/liter, and bacterial concentrations were 10^9, 10^{10}, and 10^{11} organisms/liter. The compound degradation rate was first order, as shown by the graph of concentration versus time in Fig. 1; pseudo-first-order rate constants were proportional to bacterial concentrations (Table 1). Statistical analysis of the bacteria-pesticide data showed no significant differences in rate constants between

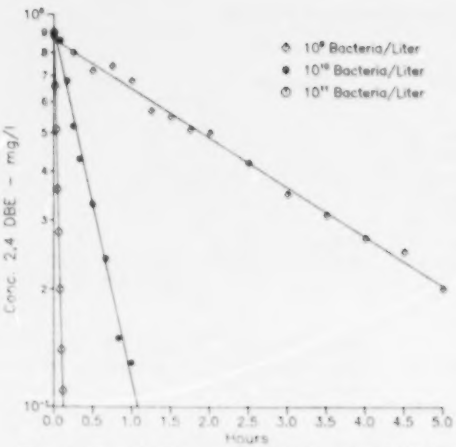

FIG. 1. *Pseudo-first-order plot for microbial hydrolysis of 2,4-DBE. Concentrations of bacteria from Overlook Lake were* 1.8×10^{7}, 1.4×10^{10}, *and* 2.1×10^{9} *organisms/liter.*

TABLE 1. *Rate constants for different concentrations of bacteria and 2,4-DBE*

2,4-DBE concn (mg/liter)	Bacterial concn (organisms/liter)	Rate constant	
		K_1 (h^{-1})	k (liters organism^{-1} h^{-1})
1.0	1.8×10^{11}	18.5	1.0×10^{-10}
	1.4×10^{10}	1.92	1.4×10^{-10}
	2.1×10^{9}	0.297	1.4×10^{-10}
0.1	2.0×10^{11}	34.8	1.7×10^{-10}
	2.6×10^{10}	4.22	1.6×10^{-10}
	2.9×10^{9}	0.517	1.8×10^{-10}
0.01	1.8×10^{11}	19.4	1.1×10^{-10}
	4.1×10^{10}	7.71	1.9×10^{-10}
	1.9×10^{9}	0.563	2.9×10^{-10}

either bacterial or pesticide levels at the 95% confidence level.

Rate constants for degradation of 2,4-DBE, malathion, and CIPC. Malathion, 2,4-DBE, and CIPC were chosen for this study because reports in the literature (1, 4, 12, 14) and studies in our laboratory (7, 8, 17) indicate that these compounds are readily degraded by hydrolytic enzymes constitutive of most bacteria in the environment. 2,4-Dichlorophenoxyacetic acid, malathion β-monoacid, and 3-chloroaniline were the major degradation products of 2,4-DBE, malathion, and CIPC, respectively (Fig. 2). Rate constants determined in these studies were for microbial hydrolysis of the parent compounds.

Sites for the water samples were selected without regard to any special characteristics. Table 2 shows the characteristics of the waters. Bacterial concentrations ranged from 4×10^{5} to 9×10^{9} organisms/liter. Dominant bacterial genera (in decreasing order) were *Pseudomonas, Alcaligenes, Serratia, Flavobacterium, Xanthomonas, Citrobacter, Enterobacter, Chromobacterium, Moraxella*, and *Yersinia*. A few *Proteus, Acinetobacter, Aeromonas, Bacillus*, and *Agrobacterium* organisms were present.

Table 3 shows rate constants for microbial transformation of 2,4-DBE, malathion, and CIPC. The value for each site is the mean of eight determinations. Duplicate flasks for the two pollutant concentrations were used in each experiment, and the experiment was repeated. Data from the sterile controls showed that abiotic hydrolysis for malathion and CIPC was negligible under the experimental conditions used. This finding is consistent with the observations of Wolfe et al. (16) on the kinetics of hydrolysis. In the few cases where abiotic hydrolysis of 2,4-DBE occurred, this contribution

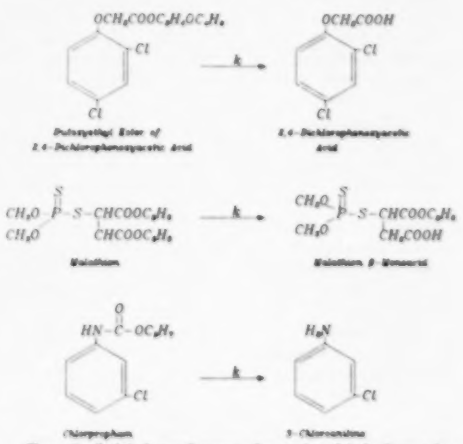

FIG. 2. *Initial pathways for microbial degradation of 2,4-DBE, malathion, and CIPC.*

TABLE 2. *Characteristics of test waters*

Characteristic	Range
Temp (°C)	1–29
pH	5.4–8.2
Total hardness (mg of $CaCO_3$/liter)	10–420
Total N (mg/liter)	Trace–10
Total P (mg/liter)	0.02–1.0
Total organic carbon (mg/liter)	1.6–28.8
Bacteria/liter	4.8×10^{5}–9.8×10^{9}
Salinity (3 waters only) (mg/10^{3})	9–24

TABLE 3. *Second-order microbial degradation rate constants*

Site	Temp (°C)	k (liters organism^{-1} h^{-1})		
		2,4-DBE	Malathion	CIPC
Northeast				
John's Creek	19	$(2.4 \pm 1.0) \times 10^{-10}$		
Potts Mt. Creek	20	$(8.6 \pm 4.5) \times 10^{-10}$		
Roanoke River	21	$(3.9 \pm 2.2) \times 10^{-10}$		
Augusta Creek	10	$(6.5 \pm 1.8) \times 10^{-10}$		
Sautatuck River[a]	10	$(1.5 \pm 0.3) \times 10^{-10}$		
Carnegie Lake	2	$(1.6 \pm 0.4) \times 10^{-10}$	$(2.9 \pm 1.1) \times 10^{-11}$	
Oneida Lake	16	$(6.7 \pm 2.1) \times 10^{-10}$	$(6.2 \pm 3.7) \times 10^{-11}$	$(1.8 \pm 1.3) \times 10^{-14}$
Princeton Canal	7			$(3.0 \pm 2.3) \times 10^{-14}$
Southeast				
Hickory Hills Pond	26		$(5.5 \pm 3.3) \times 10^{-11}$	$(1.7 \pm 1.2) \times 10^{-14}$
Oconee River	20	$(3.1 \pm 1.9) \times 10^{-10}$	$(5.3 \pm 2.9) \times 10^{-11}$	$(1.3 \pm 1.0) \times 10^{-14}$
Black Warrior River	22		$(5.9 \pm 2.3) \times 10^{-11}$	
U.S. Department of Agriculture pond	25	$(5.0 \pm 2.7) \times 10^{-10}$		
Weiss Reservoir	27		$(2.7 \pm 2.0) \times 10^{-11}$	
Manchester Creek	19	$(4.7 \pm 3.2) \times 10^{-10}$		
Shoals Creek	20	$(6.7 \pm 4.6) \times 10^{-10}$		
Richland Creek	17	$(6.4 \pm 2.2) \times 10^{-10}$		
Overlook Lake	25	$(1.6 \pm 0.6) \times 10^{-10}$	$(2.5 \pm 1.4) \times 10^{-11}$	
Okefenokee Swamp	20	$(7.6 \pm 4.7) \times 10^{-10}$		
Walker's Prong	26	$(11.5 \pm 8.9) \times 10^{-10}$		
Mississippi River	18	$(12.0 \pm 5.6) \times 10^{-10}$		$(2.0 \pm 1.6) \times 10^{-14}$
Guntersville Lake	29	$(5.9 \pm 2.0) \times 10^{-10}$		
Escambia Bay[a]	24		$(6.3 \pm 3.2) \times 10^{-11}$	
Coweeta Creek	17	$(5.2 \pm 2.7) \times 10^{-10}$		$(2.2 \pm 1.6) \times 10^{-14}$
Coosa River	28			$(2.1 \pm 1.5) \times 10^{-14}$
Wheeler's Lake	27	$(4.9 \pm 1.4) \times 10^{-10}$		
Tennessee River	27	$(1.7 \pm 0.6) \times 10^{-10}$	$(3.6 \pm 2.9) \times 10^{-11}$	
Gulf Breeze[a]	27	$(5.6 \pm 2.9) \times 10^{-10}$		
Northwest				
Willamette River	20			$(2.9 \pm 2.1) \times 10^{-14}$
Duluth water treatment	17	$(5.4 \pm 1.0) \times 10^{-10}$		
St. Louis River	18	$(6.0 \pm 1.7) \times 10^{-10}$		
Pond Oregon	20	$(8.0 \pm 1.3) \times 10^{-10}$		
Columbia River	20	$(6.8 \pm 2.6) \times 10^{-10}$	$(4.2 \pm 3.6) \times 10^{-11}$	
Blue River (summer)	26	$(37 \pm 12) \times 10^{-10}$[b]	$(5.2 \pm 1.0) \times 10^{-11}$	
Blue River (winter)	1	$(7.1 \pm 6.3) \times 10^{-10}$		
Lake Superior	16		$(4.9 \pm 3.0) \times 10^{-11}$	$(4.9 \pm 4.1) \times 10^{-14}$
Southwest				
Ada	20	$(6.3 \pm 3.1) \times 10^{-10}$		$(3.1 \pm 2.1) \times 10^{-14}$
Searsville	15	$(5.5 \pm 1.8) \times 10^{-10}$		
Trinity River	12	$(3.6 \pm 1.6) \times 10^{-10}$		
Lake Travis	22	$(2.3 \pm 1.3) \times 10^{-10}$	$(4.0 \pm 3.0) \times 10^{-11}$	$(3.9 \pm 1.8) \times 10^{-14}$
Lake Mead	22	$(3.8 \pm 1.5) \times 10^{-10}$	$(4.4 \pm 2.2) \times 10^{-11}$	
Mean	19.5 ± 6.7	$(5.4 \pm 2.7) \times 10^{-10}$	$(4.4 \pm 2.9) \times 10^{-11}$	$(2.58 \pm 1.29) \times 10^{-14}$

[a] Marine waters.
[b] Not included in mean value or statistical analysis.

(0 to 15% of the 2,4-DBE hydrolyzed in the sterile controls) was subtracted.

2,4-DBE was more readily degraded than malathion, and malathion was more readily degraded than CIPC. Using mean values of degradation rate constants [$(5.4 \pm 2.7) \times 10^{-10}$ liter/organisms per hour for 2,4-DBE, $(4.4 \pm 2.9) \times 10^{-11}$ for malathion, and $(2.6 \pm 1.3) \times 10^{-14}$ for CIPC] and a bacterial concentration of 5×10^8 organisms/liter, the half-lives would be 2.6, 32, and 53,000 h for 2,4-DBE, malathion, and CIPC, respectively. Because of the long half-life of CIPC, the waters with 0.2 g per liter of nutrient broth were incubated for 48 h to build up high

bacterial populations before experiments were begun. Experiments with three waters and no nutrient broth were continued through almost one half-life. The experiments were replicated, and nutrient broth was added as described. Results indicated no discernible difference between rate constants determined in waters with the different bacterial concentrations.

Statistical analysis of the kinetics data included analyses of variance that provided tests of significance for site and concentration effects. The experimental design included replication and site as random effects, concentration and region as fixed effects, and interaction terms. Of particular interest were (i) whether the variance among sites was zero and (ii) whether the effects of concentration were the same at the levels selected.

Table 4 summarizes the results of the various significance tests by procedures appropriate for the statistical model. For convenience in detecting changes among sites, the United States was divided arbitrarily into four regions: northeast, southeast, northwest, and southwest. The "among sites" sum of squares may be decomposed into "among regions" and "among sites within regions," and the latter may be broken down for the individual regions. The significance tests indicated no difference among sites for the three compounds at the 0.05 level. Similar decompositions or breakdowns for other sources are possible but, for brevity, are omitted from the table. The table also contains estimates of the site-to-site variances and the overall error variance (mean square error) or the within-site variance for each compound.

DISCUSSION

In the study with Overlook Lake bacteria and 2,4-DBE, high concentrations of bacteria were used, and the bacterial concentration remained approximately constant. Xenobiotic degradation was first order; i.e., a straight line was obtained when log [S] was plotted versus t (Fig. 1). The order of the reaction with respect to the bacteria was demonstrated by showing that the reaction rates were proportional to the bacterial concentrations. Similar experiments with malathion and CIPC were not performed. With data from the site-to-site study, however, graphs (Fig. 3 and 4) of log of malathion or CIPC concentrations versus time gave straight lines; rates of degradation were proportional to the bacterial concentrations.

TABLE 4. *Summary of results of significant tests*

Source of variation	Significance		
	2,4-DBE	Malathion	CIPC
Replication	NS[a]	NS	NS
Among sites	NS (3.642)	NS (~0)	NS (0.349)
Region	NS	NS	NS
Site (region)	NS	NS	NS
Northeast	NS (3.84)	S[b] (5.01)	NS (0.3)
Southeast	NS (5.54)	NS (0.76)	NS (0.06)
Northwest	NS (0.625)	NS (~0)	S (3.76)
Southwest	NS	NS (~0)	NS (0)
Concn	NS	NS	NS
Residual	2.17	3.52	1.92

[a] NS, Not significant at the 0.05 level.

[b] S, Significant at the 0.05 level. Numerical entries are variance component estimates.

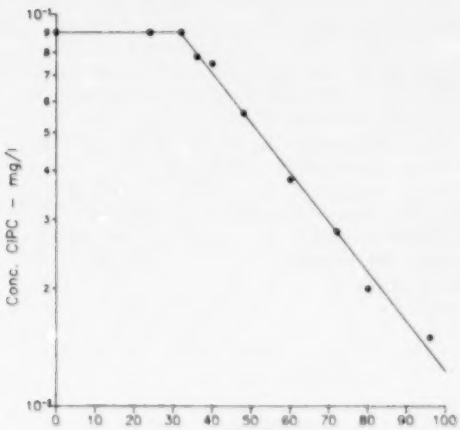

FIG. 3. *Pseudo-first-order plot for microbial hydrolysis of CIPC. The concentration of bacteria from Hickory Hills Pond was* 1.7×10^{13} *organisms/liter.*

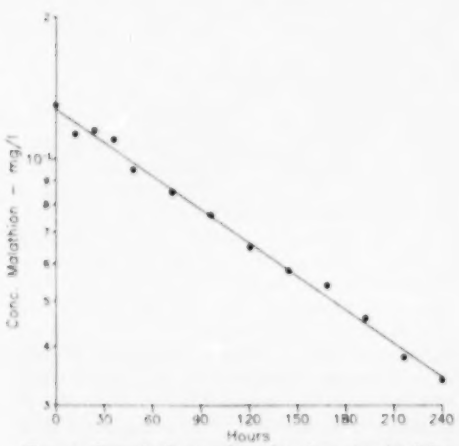

FIG. 4. *Pseudo-first-order plot for microbial hydrolysis of malathion. The concentration of bacteria from Oneida Lake was* 1.9×10^{8} *organisms/liter.*

Of the two parameters measured for the model, an accurate estimate of the bacterial population was the more difficult to obtain. The standard plate count technique was used to determine bacterial concentrations. In earlier laboratory degradation studies (7) in which the xenobiotic was the sole carbon source, plate counts appeared to be a satisfactory measurement of the bacterial population. In those studies and in the report of Witkamp (15), good correlations between decrease of substrates and bacterial populations measured by plate counts have been shown. Other determinations, like dry weight or adenosine triphosphate, are difficult to use for environmental studies because of low levels of microbial biomass in natural waters.

Data developed in this study suggest that a second-order rate equation can be used to describe microbial transformation of xenobiotics at low concentrations. The techniques used were not new, but expressing results as rate constants that are presumably independent of site is a different approach. The rate constants (k) for the given compounds were reproducible (±65%) for different sites. Using these constants for predicting the microbial fate of xenobiotics, one would obtain good approximations of what can happen in the environment. Only one rate constant (2,4-DBE in summer Blue River) was not in good agreement with the other values (Table 3). The sample water was light green and contained diatoms and algae in numbers large enough that, if they had enzymes that degraded 2,4-DBE at the same rate as the bacteria, they could have affected the rate constant significantly. The rate constant for microbial degradation of malathion in the same water sample, however, agreed with values for other sites.

Results of our studies suggest that the second-order approach can be used in environmental fate studies. At concentrations normally encountered in rivers and lakes, rate constants were within a factor of eight and independent of the site from which the water was taken. Such variation seems reasonable considering the variability associated with individual environmental parameter estimates (Table 2). Good agreement among rate constants was found not only within this study, but also in a comparison of these data with data from an earlier laboratory study (7). Earlier rate constants for malathion and 2,4-DBE degradation (7) were within an order of magnitude of the constants determined in this study, although experimental conditions were quite different. No explanation is apparent. If the rate constants for other compounds and different sites can be shown to be in good agreement, the approach will provide a useful tool for approximating the microbial fate of xenobiotics.

Probably the greatest potential use of this approach is in the area of exposure assessment modeling. In the absence of detailed microbial studies of aquatic ecosystems, application of this model could be a reasonable approach. Such models provide information for engineers and scientists to make rational decisions relevant to the fate of toxic compounds. Baughman and Burns (2) have recently reviewed such environmental assessment modeling capabilities.

For the purposes of this study, water characteristics that may affect the rate constants were not addressed. Although the range of values among our samples for these characteristics was wide, many values were clustered, as shown for temperatures in Table 3, and were not distributed properly to evaluate their role. The differences in water characteristics could account for part of the 65% relative standard deviation reported. Nonetheless, the deviation is low and consistency is good considering the number of sites. Agreement among rate constants from this study with heterogeneous microbial populations and natural organic substrates suggests that a similar approach can be taken with other xenobiotics.

Our hypothesis for these studies, i.e., that the second-order rate constant for microbial hydrolysis of xenobiotics would be reproducible from site to site, has been confirmed. Although more data are needed with different sites and different compounds, these results do offer significant encouragement for prediction of microbial kinetics in natural systems.

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